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Cercaria kuntzi n. sp. (Trematoda) from *Oncomelania hupensis formosana*, Its Metacercariae and Chromosomes

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Chin-Tsong Lo and Kin-Mu Lee (1996) *Cercaria kuntzi* n. sp. (Trematoda) from *Oncomelania hupensis formosana*, its metacercariae and chromosomes. *Zoological Studies* **35**(4): 255-260. *Cercaria kuntzi* n. sp. parasitic to *Oncomelania hupensis formosana* is described from Taiwan. It has a continuous dorso-ventral finfold on the tail and is biocellate, the left eyespot being double which becomes apparent when pressed. The cercariae penetrated the tadpoles of *Rana latouchi* and became mature metacercariae in a month. The metacercariae excysted and survived for 2 weeks in a species of *Natrix* snake without growth or differentiation, but this suggests that its final hosts are probably poikilothermic vertebrates. It has a diploid chromosome number of 14 consisting of 7 homologous pairs. Possible benefit of chromosomal information in the classification of cercariae is discussed.

Key words: Trematoda, *Cercaria kuntzi* n. sp., *Oncomelania hupensis formosana*, Chromosome number, Cercaria.

Up to the present, 13 species of cercariae have been described from the snail, *Oncomelania hupensis*, occurring in Japan, China, Taiwan, and the Philippines (Ito 1964 1988, Ito et al. 1977). They include 3 furcocercariae, one of which is *Schistosoma japonicum*; 5 xiphidiocercariae, one of which is *Maritrema caridinae*; 3 microcercous cercariae (lung flukes *Paragonimus ohirai*, *P. iloktsuenensis*, and *P. miyazakii*), and 2 gymnocephalous cercariae one of which is trioculate. From *O. h. formosana* (Fig. 1), the subspecies that occurs in Taiwan, only 2 species of cercariae have been reported, namely *S. japonicum* and *M. caridinae* (Yokogawa 1915, Kuntz 1965, Lo 1990). During the past several years an undescribed cercaria has been observed emerging from *O. h. formosana*, and it is reported here for the first time. Metacercariae were obtained by means of experimental infections and this species' chromosome number and karyotypes were also studied.

MATERIALS AND METHODS

Snails infected with this parasite were found from several localities in 2 counties separated by a distance of about 100 km: Fenshulin and Shuili of Nantou County in central Taiwan, and Chienshan and Liukuei of Kaohsiung County in the south. Several collections exhibited infection rates of less than 1%. The snails used in this study were collected from the Liukuei locality. Metacercariae were obtained by exposing cercariae to tadpoles of *Rana latouchi* which were later dissected. Rediae were obtained by gently crushing the snail, and then dissecting it under a stereoscope. The cercariae and rediae were observed alive in 0.2%-0.4% NaCl, as well as after fixation in hot 10% formalin and staining with acetocarmine. The chromosome number and karyotype were studied by the squash procedures (La Cour 1941) using the cercarial embryos obtained from the rediae. Sizes of anatomical structures were measured on 10 fixed specimens and their means and ranges are reported. All measurements reported here are in micrometers (μm). In addition to the tadpoles, 4 species of freshwater fish (*Gambusia affinis*,

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Cyprinus carpio, *Zacco platypus*, *Misgurnus anguillicaudatus*) were exposed to 100 cercariae to see if these fish were capable of serving as the 2nd intermediate host. A preliminary study was also carried out to determine the definitive host by feeding 10 metacercariae each to 1-2 laboratory mice; domestic ducks; domestic chickens; munias, *Lonchura* sp.; and snakes, *Natrix* sp., representing both poikilotherms and homoiotherms. Syntypes of the cercariae, rediae and metacercariae have been deposited in the U.S. National Parasite Collection, USDA, Beltsville, Maryland 20705, USA.

RESULTS

Description

Cercaria (Figs. 2, 7): Light brown in color owing to brown pigment particles scattered in the body, positively phototactic, swimming continuously until exhaustion, some surviving for 2 days at 24 to 26 °C. Biocellate, with a single right ocellus and a double left ocellus; the double nature of left ocellus more apparent when pressed. Body 225 (190-255) long by 107 (100-110) wide; tail 473 (450-495) long by 37 (32-42) wide. About 30 cystogenous glands on dorsal side of body, obliterating the view of penetration glands; however, gland ducts clearly visible and grouped into 4 bundles of 3-4-4-3 near the anterior end and open at dorsal front edge of oral sucker. Oral sucker 39 (38-50) long by 36 (33-40) wide; ventral sucker vestigial, at anterior 2/3 of body, consisting of a cell mass 23-25 in diameter. Prepharynx short. Pharynx, 15-20 in diameter, followed by a long slender esophagus and short ceca. V-shaped cellular excretory vesicle containing fine granules. Flame cell number and arrangement not able to be accurately determined, but up to 24 cells counted on each side. Anterior body surface covered with spines, denser towards the anterior end and disappearing at the level of eyespots. About 12-14 sensory hairs on each side of body consisting of long (8-12) and short (1-2) types. Each sensory hair arising from a papilla. Tail pleurolophocercous type, 473 (450-495) long by 37 (30-40) wide; dorsal finfold arising at proximal 2/5, continuing around tail tip into ventral finfold which extends about distal 1/3 of tail length. About 27-35 caudal bodies scattered in the tail, a half of which concentrated at posterior 1/4 where it curves towards the dorsal side.

Redia (Fig. 3): All the rediae observed contained only the cercarial embryos, thus are presumed to be daughter rediae. Sausage-shaped, motile in 0.4% saline, without pigment, collar or locomotive appendages, containing 12-16 cercarial embryos in the anterior and several germinal balls in the posterior sections. Nearly same width throughout; length 435 (390-480), width 110 (90-140). Pharynx 32 (28-35) long by 32 (29-35) wide. Intestine 28 (20-33) by 32 (21-38), obliquely behind pharynx, spherical to oval in shape, devoid of food particles but with a few refractile granules. No mature cercariae were seen inside the redia, while both mature and immature cercariae were present in the blood space of the snail. Birth pore immediately posterior to pharynx. Numbers of rediae in each snail (n = 5) ranged from 32 to 84.

Metacercaria (Figs. 4, 5): Mature cysts at 40 days postinfection (DPI) slightly oval, 320 by 300, with a thin wall of 1, and without eyespots. Excretory vesicle distinct, with black contents extending anteriorly as far as posterior margin of oral sucker; contents dissolved in acetic acid. Excysted and flattened metacercariae at 40 DPI 567 (525-610) long by 242 (220-280) wide; oral sucker 106 (88-128) long by 114 (98-135) wide; ventral sucker at midbody, 98 (90-108) long by 107 (93-120) wide; pharynx 42 (40-45) long by 34 (28-35) wide. Noodle-like threads in ceca.

Chromosomes (Fig. 6): Mitotic chromosomes at metaphase with diploid number of 14 consisting of 7 homologous pairs. The 7 pairs are classified into 4 groups by length, in the order of decreasing length: 2 exceptionally long and submetacentric; 1 with a satellite and submetacentric; 3 of similar size; 2 of which submetacentric and 1 subtelocentric; and the shortest, a submetacentric chromosome. The ratios of chromosome length of the 7 pairs measured on early metaphase plates (n = 6) were: 1, 0.72, 0.24, 0.22, 0.22, 0.18, and 0.10, respectively.

Taxonomic summary

Definitive host: Unknown.

First intermediate host: *Oncomelania hupensis formosana*.

Second intermediate host: Tadpoles of *Rana latouchi* (Experimental infection).

Locality: Liukuei, Kaohsiung County, Taiwan (23°01'N, 120°37'E).

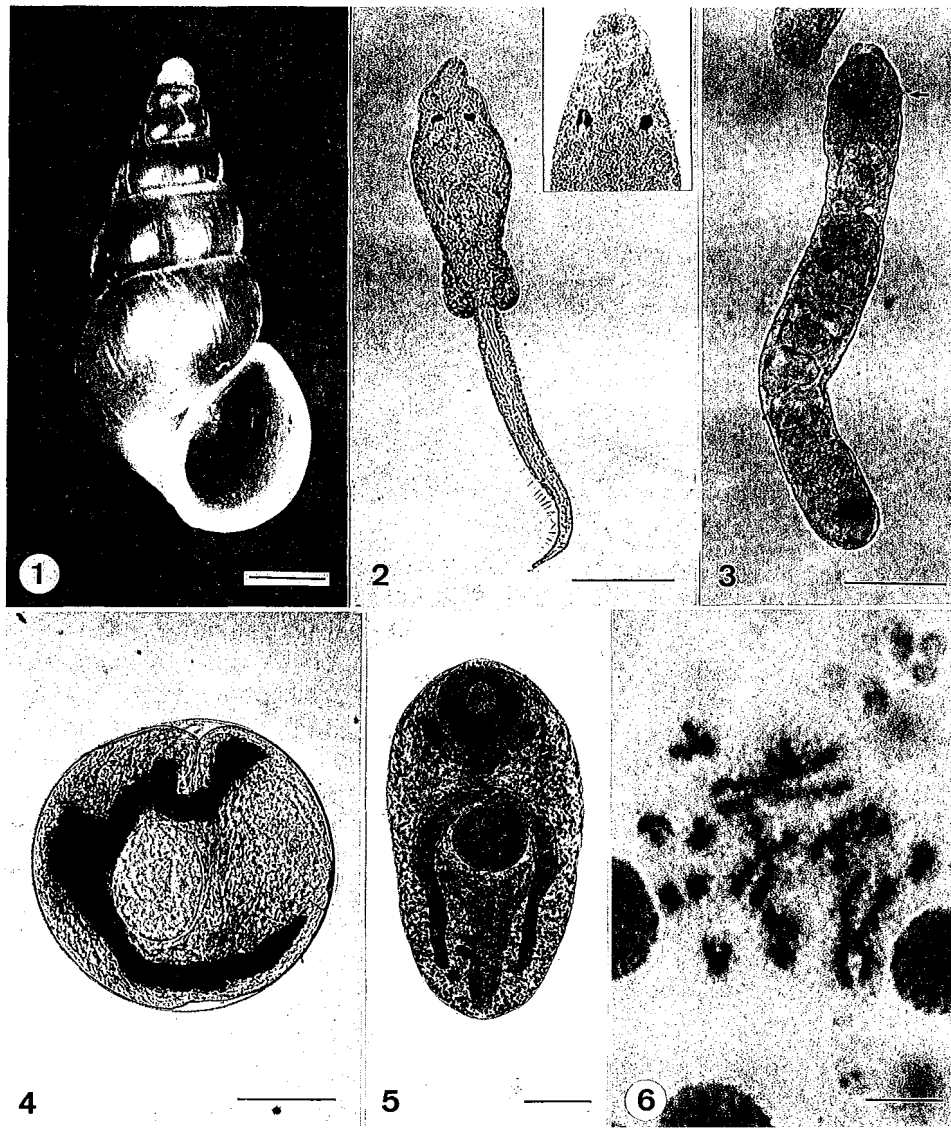
Specimens deposited: Syntypes stained with acetocarmine-fast green, and mounted in Permount®. Six cercariae (USNPC No. 85962), 3 rediae (USNPC No. 85963), 3 excysted metacercariae

(USNPC No. 85964).

Etymology: The species is named after Dr. Robert E. Kuntz, formerly of the U. S. Naval Medical Research Unit No. 2, who contributed greatly to the knowledge of digenean fauna of Taiwan.

Life cycle studies

Second intermediate host: The cercariae readily penetrated all parts of the body of tadpoles of *Rana latouchi*, migrated, and encysted within a day, with the majority in the head region. The shortest time for penetration was 3 min, with the majority requiring 5-7 min and some taking up to 12 min. Cyst recovery rates in some tadpoles were higher than 50%. By 20 DPI, a distinct ventral sucker had developed, the eyespots had disappeared in most specimens, and genital primordia could be recognized in the hindbody (Fig. 5). By 30 DPI the metacercariae appeared to be fully mature since there was no further growth. In the 4 fish species tested, the cercariae tried to penetrate also, but 10 min after exposure many of them had lost their tails and sunk to the bottom, an



Figs. 1-6. Photographs of the host snail and some life cycle stages of *Cercaria kuntzi* n. sp.
1. Shell of *Oncomelania hupensis formosana*. 2. Live cercaria. Inset: anterior part of a pressed specimen to show the double left ocellus. 3. Live redia. Arrow indicates the birth pore. 4. Live encysted metacercaria at 40 DPI. 5. Excysted metacercaria at 40 DPI, acetocarmine stained. 6. Mitotic chromosomes at early metaphase. Bar = 1 mm in Fig. 1; 100 μm in Figs. 2-5; 5 μm in Fig. 6.

indication of penetration failure. In the case of *G. affinis*, which swims in surface waters, about a half of the cercariae were still swimming as usual at 3 h post-exposure indicating that they had not yet contacted the fish. Less than 2% of the cercariae formed cysts on the body surface of the fish, but they were somewhat deformed and eventually dropped off and died. A few cercariae successfully penetrated the fin of *M. anguillicaudatus*, but their fates were not followed.

Definitive host: No worms were recovered from laboratory mice, ducks, chickens, or *Lonchura* sp. when examined 1-2 wk after feeding with the metacercarial cysts. From *Natrix* sp. 2 worms were recovered, but they showed little growth or differentiation.

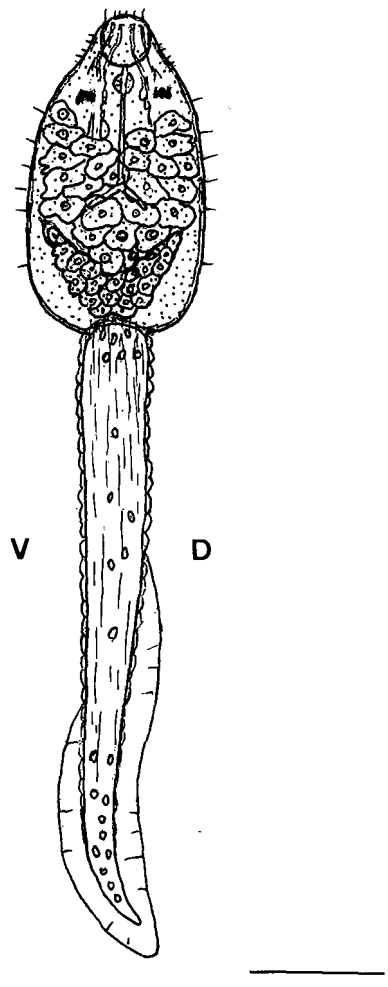


Fig. 7. Composite drawing of *Cercaria kuntzi* n. sp. Body is a dorsal view while tail is a side view. D, dorsal side; V, ventral side. See text for explanation. Bar = 100 μm.

DISCUSSION

The cercaria reported here belongs to the pleurolophocercous group. According to Schell (1970), the cercariae of this group develop in operculate snails and encyst in fish. However, the results of our penetration study suggested that fish would be very poor, if not impossible, 2nd intermediate hosts. Furthermore, the snail hosts were collected from roadside ditches with little water which would be dry for several months during a year and in which no fish were present. The recovery of young worms from snakes suggests its final hosts are probably cold-blooded vertebrates.

Biocellate pleurolopho-cercariae occur in some members of several families, such as *Clonorchis sinensis* and *Opisthorchis viverrini* of the Opisthorchiidae (Komiya and Tajimi 1940a, Wykoff et al. 1965), *Stellantchasmus falcatus* and *Metagonimus yokogawai* of the Heterophyidae (Takahashi 1929, Martin 1958), and *Exorchis oviformis* and *Pseudexorchis major* of the Cryptogonimidae (Komiya and Tajimi 1940b, Ito 1956). Comparison of cercariae or rediae of these species with *Cercaria kuntzi* n. sp. easily revealed differences between them in behavior, coloration, anatomical structures, or combinations thereof. For example, the daughter rediae of *C. sinensis* are twice as long, the cercariae have neither esophagus nor ceca, and the sensory hairs on the body are much longer. The cercariae of *E. oviformis* are transparent, without an esophagus or ceca, and the dorso-ventral fins are present along the entire tail. The cercariae of *S. falcatus* are much smaller especially the body, the dorsal fin begins at the base of tail and the intestine of the redia is elongated. In addition, the host snails of the 3 cercariae compared above all belong to different families than *O. h. formosana*.

Some morphological and developmental characters seen in *C. kuntzi* n. sp. are also shared by various groups of digeneans. For example, the double left ocellus and a single right one also occur in cercariae of *S. falcatus*, *Centrocestus formosanus*, and *Haplorchis taichui* (Martin 1958); the completion of cercarial maturation in the snail's tissue space rather than inside the redia has been observed in *C. sinensis*, *E. oviformis*, *P. major*, and *Cryptocotyle lingua* (Stunkard 1930, Komiya and Tajimi 1940a, b, Ito 1956). Although the penetration glands proper could not be observed in *C. kuntzi*, it probably has 7 pairs judging from the number of gland ducts. The number of penetration glands varies greatly even within a family, thus 4, 7, and 9 pairs have been reported for the Hetero-

phyidae; 5, 7, and 10 pairs for the Opisthorchiidae, and 7 pairs for the Cryptogonimidae (Yamaguti 1975).

Chromosome number of 2n = 14 have been reported in diverse groups of digeneans, e.g., Paramphistomatidae, Notocotylidae, Allocreadiidae, Gorgoderidae, Schistosomatidae (review by Walton 1959), and Fasciolidae (Lo 1969), indicating that the number per se is not useful in the classification of digeneans at higher categories. However, the chromosome number determination combined with karyotype analysis might be useful at the species level within a genus or family, and would help in the elucidation of life cycle, because cercariae from heterogenous groups often possess common adaptive characters making it difficult to distinguish between them, while chromosome numbers and karyotypes are conservative.

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感染於臺灣釘螺之吸蟲尾動幼蟲之一新種 *Cercaria kuntzi* n. sp.

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我們發現從臺灣釘螺游出一種新的尾動幼蟲 (尾幼)，而將其命名爲 *Cercaria kuntzi* n. sp. 以紀念孔志博士 (Dr. Robert E. Kuntz)。感染之釘螺發現自南投縣之水里及楓樹林，以及高雄縣之六龜及建山。此尾幼有背腹鰭及兩個眼點，自釘螺釋出後不停地游動至死爲止。拉杜希氏蛙 (*Rana latouchi*) 之蝌蚪可當做其第二中間宿主，尾幼穿入其體內後，經一個月左右時間發育爲成熟的囊狀幼蟲 (囊蟲)，所試過之四種淡水魚 (鯉魚、大肚魚、小溪哥、泥鰍) 爲不適當的第二中間宿主，因爲絕大部分之尾幼未能穿入。爲了取得成蟲以便完成其生活史，我們將囊幼餵食給小白鼠、鴨子、雞以及文鳥，但不成功。如果餵食給一種水蛇 (*Natrix* sp.)，則取到了幼蟲，但這些幼蟲並沒有成長或器官之分化；然而此結果暗示其終宿主可能是冷血脊椎動物。以尾幼胚胎爲材料檢查有絲分裂的結果得知牠有14條染色體，並可排成7對。我們認爲染色體之研究對尾幼之分類應有一定的幫助，而且也是新的嘗試。

關鍵詞：吸蟲，尾動幼蟲，新種，染色體，臺灣釘螺。

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